oxygen, salinity, and temperature were collected using Yellow Springs Instrument (YSI) Inc. Model 85 water quality meters. Near-surface measures of pH were collected using a pHep® 3 field microprocessor meter. More extensive time-profile measurements of all four parameters were obtained from the near-bottom waters of each site using YSI Model 6920 multiprobes logging at 15 min intervals for a minimum of 25 hrs to assess conditions over two full tidal cycles representing both day and night conditions.

Water quality samples included near-surface measures of nitrogen (including ammonia, nitrate/nitrite, total Kjeldahl nitrogen (TKN), and total nitrogen (TN)), total phosphorus (TP), total organic carbon (TOC), total suspended solids (TSS), turbidity, five-day biochemical oxygen demand (BOD_5), chlorophyll-*a*, and fecal coliform bacteria concentrations. Near-surface measures of dissolved nutrients, including ammonia, inorganic nitrogen (DIN), organic nitrogen (DON), inorganic phosphorus (orthophosphate or OP), organic phosphorous (DOP), and silica (DS), were also collected. All samples were collected by inserting pre-cleaned water bottles to a depth of 0.3 m inverting and then filling the bottle directly at that depth. Water samples collected for dissolved nutrient quantification were filtered in the field through a 0.45 µm pore cellulose acetate filter. The bottles were then stored on ice until they were returned to the laboratory for further processing. Total nutrients, TOC, total alkalinity, TSS, turbidity, BOD_5, chlorophyll-*a*, and fecal coliform bacteria samples were processed by SCDHEC using standardized procedures (SCDHEC, 1998b, 2001, 2005). Dissolved nutrients were processed through the University of South Carolina using a Technicon AutoAnalyzer and standardized procedures described by Lewitus *et al.* (2003). DON and DOP were calculated by subtracting total inorganic from total dissolved N or P, measured by the persulfate oxidation technique (D'Elia *et al.,* 1977).

2.3. Biological and Sediment Sampling

Bottom sediment samples were collected at each station using a stainless steel 0.04 m^2 Young grab deployed from an anchored boat. The boat was repositioned between each sample to ensure that the same bottom was not sampled twice and to spread the samples over a 10-20 m^2 bottom area. The grab was thoroughly cleaned prior to field sampling and rinsed with isopropyl alcohol between stations. Three of the grab samples were washed through a 0.5 mm sieve to collect the benthic invertebrate fauna which were then preserved in a 10% buffered formalin-seawater solution containing rose bengal stain. The surficial sediments (upper 3 cm) of the remaining grab samples were homogenized on site and placed in pre-cleaned bottles for analysis of sediment composition, contaminants, and sediment toxicity. All sediment samples were kept on ice while in the field and then stored either at 4°C (toxicity, porewater) or frozen (contaminants, sediment composition, TOC) until analyzed.

The Young "grab" is used to collect sediments and benthic fauna. Photo credit: R. Van Dolah

Particle size analyses were performed using a modification of the pipette method described by Plumb (1981). Pore water ammonia was measured using a Hach Model 700 colorimeter and TOC was measured on a Perkin Elmer Model 2400 CHNS Analyzer.

Contaminants measured in the sediments included 22 metals, 25 polycyclic aromatic hydrocarbons (PAHs), 79 polychlorinated biphenyls (PCBs), 13 polybrominated diphenyl ethers (PBDEs), and 21 pesticides. All contaminants were analyzed by the NOAA-NOS Center for Coastal Environmental Health and Biomolecular Research (CCEHBR) using procedures similar to those described by Krahn *et al.* (1988), Fortner *et al.* (1996), Kucklick *et al.* (1997), and Long *et al.* (1997).

Sediment toxicity was measured using three bioassays. They included: (1) the Microtox® assay using a photoluminescent bacterium, *Vibrio fischeri*, and protocols described by the Microbics Corporation (1992), (2) a 7-day juvenile clam growth assay using *Mercenaria mercenaria* and protocols described by Ringwood and Keppler (1998), and (3) a 10-day whole sediment amphipod assay using *Ampelisca abdita* and protocols described by ASTM (1993). Toxicity in the Microtox® assay was based on criteria described by Ringwood *et al.* (1997; citerion #6: toxic when scores of < 0.5 if silt/clay < 20% and scores of < 0.2 if silt/clay > 20%). For the clam assay, sediments were considered toxic if growth (dry weight) was < 80% of that observed in control sediments and there was a statistically significant difference ($p < 0.05$). For the amphipod assay, sediments were considered toxic if survival was < 80% of that observed in control sediments and the difference was statistically significant ($p < 0.05$).

Water samples for phytoplankton community analysis were collected from near-surface water concurrently with water quality samples. Fresh samples were examined under a microscope for species identifications, and subsamples were filtered and analyzed for taxon-specific biomass determination. While chlorophyll-*a* is a useful surrogate for computing phytoplankton biomass, it must be coupled with species-specific pigment ratios to yield information about community composition. This analytical method, CHEMTAX, is a matrix factorization program that generates a profile of the community based on the pigment ratio detected in the water sample using High Pressure Liquid Chromatography (HPLC) (Lewitus *et al.*, 2005). HPLC data can be used to calculate the portion of the phytoplankton community attributable to individual taxonomic groups. It is not as refined as counting individual species of phytoplankton, but it allows for rapid and accurate quantification of biomass of relevant groups of phytoplankton. Using these pigment ratios, the community can be divided into species which are typically present in a pristine estuarine environment (diatoms, mixed flagellates) versus those which are abundant in nutrient-rich seawater (dinoflagellates, raphidophytes) or nutrient-rich freshwater inflows (cyanobacteria).

Two of the three grab samples collected to assess benthic invertebrate community composition were sorted in the laboratory to separate organisms from the sediment remaining in the sample. The third grab sample was held in reserve. All organisms from the two grabs were identified to the species level or to the lowest practical taxonomic level possible if the specimen was damaged or too immature for accurate identification. A reference collection of all benthic species collected for this program is being maintained at the SCDNR Marine Resources Research Institute.

Fish and large crustaceans (primarily penaeid shrimp and blue crabs) were collected at each site following benthic sampling to evaluate near-bottom community composition. Two replicate tows were made at each site using a 4-seam trawl (5.5 m foot rope, 4.6 m head rope and 1.9 cm bar mesh throughout). Trawl tow lengths were standardized to 0.5 km for open water sites and 0.25 km for creek sites. Tows were made only during daylight hours with the current, and boat speed was standardized as much as possible. Tows made in tidal creeks were limited to periods when the marsh was not flooded (approx. 3 hrs $\pm$ mean low water). This limitation was also generally applied to open water sites. Catches were sorted to lowest practical taxonomic level, counted, and checked for gross pathologies, deformities or external parasites. All organisms were measured to the nearest centimeter. When more than 25 individuals of a species were collected, the species was sub-sampled. Mean abundance of finfish and

Trawls are used to sample mobile fish and crustaceans. Photo credit: R.F. Van Dolah

crustaceans were corrected for the total area swept by the two trawls using the formula described by Krebs (1972).

Fish tissue samples for contaminant analyses were obtained from trawls. Targeted species included spot (*Leiostomus xanthurus*) and Atlantic croaker (*Micropogonias undulatus*). Silver perch (*Bairdiella chrysoura*) or weakfish (*Cynoscion regalis*) were collected if they were present when the target species were not. All fish samples were wrapped in foil and stored on ice in plastic bags until they could be frozen in the laboratory. Entire fish were then rinsed and homogenized in a stainless steel blender. Extraction and analytical procedures were similar to those described for sediments.

2.4. Habitat Evaluation

Observations were made at each site prior to departure to document the presence of litter (within the limits of the trawled area) and to note the proximity of the site to urban/suburban development or industrial development.

2.5. Quality Assurance

SCECAP protocols include rigorous quality assurance and quality control guidelines for all aspects of the program to ensure that the database is of high quality. A copy of the Quality Assurance Project Plan is maintained at the SCDNR Marine Resources Research Institute and has been approved by the USEPA NCA Program.

2.6. Data Analyses

Comparisons of most water quality, sediment quality and biological measures were completed using standard parametric tests or non-parametric tests where the values could not be transformed to meet parametric test assumptions. Two stations (RO046286 and RT042266) were not included in the comparisons, since these sites represented special study sites selected to add stations in the Charleston Harbor estuary. Comparisons of measurements collected in tidal creek versus open water habitats were conducted using a t-test or non-parametric Mann-Whitney U test. Comparisons involving more than two station groups or multiple years were generally completed using ANOVA or Kruskal-Wallis tests. Data from 2003 and 2004 were generally pooled within each habitat type to calculate the current condition of and temporal trends in most individuals measures. Data from the two years were separated within each habitat type to examine changes in integrated water quality and sediment quality scores, benthic biological condition and overall habitat quality as well as for several individual measures of particular concern.

Use of the probability-based sampling design provided an opportunity to statistically estimate, with confidence limits, the proportion of South Carolina's overall creek and open water habitat that falls within ranges of values that were selected based either on (1) state water quality criteria, (2) historical measurements collected by SCDHEC from 1993-1997 in the state's larger open water bodies (SCDHEC, 1998a), or (3) other thresholds indicative of stress based on sediment chemistry or biological condition (Hyland *et al*., 1999; Van Dolah *et al.,* 1999). These estimates were obtained through analysis of the cumulative distribution function (CDF) using procedures described by Diaz-Ramos *et al*. (1996).

3. RESULTS AND DISCUSSION

Data obtained from the 2003-2004 survey are summarized in the following sections. More extensive data summaries are also available on the SCECAP web site (http://www.dnr.sc.gov/marine/scecap/) and are referenced in this report as "data online."

3.1. Station Array

The locations of the 60 sites sampled in 2003 and 2004 are provided in Figures 3.1.1 - 3.1.4 and Appendix 1. Tidal creek station numbers are designated by RT, and open water stations are designated by RO. As noted previously, the two supplemental sites sampled in 2004 to obtain additional data for the Charleston Harbor estuary (RO046286 and RT042266) are not included in the general analyses of state-wide condition, but the data are available online.

The average depth of open water sites sampled during the two-year period was 5.2 m and varied from approximately 1.2-14.0 m (Appendix 1, data online).